

HISTOMORPHOLOGY OF SKIN EPIDERMIS OF CATTLE BREEDS OF MAHARASHTRA IN DIFFERENT CLIMATIC CONDITION

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Abstract: The present study was conducted on skin samples of 4 – 6 years of age healthy Deoni, Red kandhari, Dangi and Gaolao breeds of cattle managed under hygienic conditions on farm in different regions of Maharashtra. The skin samples, 10 of each breed were obtained surgically from loin region during winter and summer seasons separately.

The epidermis was comprised of four sub layers namely stratum corneum, stratum granulosum, stratum spinosum and stratum basale from outside in. The stratum granulosum was two cell layers in thickness in Gaolao cattle during both seasons, whereas it was single cell layer in thickness in other cattle breeds. The thickness of stratum corneum and epidermis was found more during winter season in all cattle breeds. The melanocytes and Langerhans cells were distributed among cells of stratum basale and stratum spinosum. The density of melanin pigmentation was more in basal epidermal region and was reduced towards the superficial part of epidermis. The epidermal pegs were the down growth of epidermis in the underlying dermis. The average numbers of epidermal pegs varied significantly among breeds as per climatic and geographic conditions.

Keywords: Histology, Epidermis, cattle Breeds, Climatic condition.

Material and Methods

The present study was conducted in the Department of Veterinary Anatomy and Histology, College of Veterinary and Animal Sciences, Parbhani (M.S.). The experiment was carried out on 40 female cattle of 4 – 6 years of age belonging to different breeds located in different regions of Maharashtra state during winter (November - February) and summer (March – June) seasons, separately. The skin samples, 10 of each were obtained from loin region of healthy Deoni, Red kandhari, Gaolao and Dangi breeds of cattle, managed under hygienic conditions on the farm in different regions of Maharashtra state. Tissue pieces of 5 mm size were cut to preserve in following fixatives for the histomorphological study.

1. 10% Neutral buffered formalin
2. 10% formalin
3. Bouin's fluid

After fixation, tissues were washed in running tap water for overnight. These were then processed for routine paraffin technique. The tissues were first passed through ascending

grades of alcohol, cleared in xylene, infiltrated in three changes of paraffin (melting point 580-600C) and then embedded in paraffin by employing manual tissue processing schedule suggested by Drury and Wallington (1980).

The longitudinal and transverse tissue sections of 4 to 5 μ thickness were obtained on manually operated rotary microtome. The sections were mounted on glass slides and dried at room temperature for 24 hours and were preserved carefully for staining. The following staining methods were used for histomorphological studies.

- a) Harri's Haematoxylin and Eosin stain for normal histoarchitectural study (Mukharjee, 1992).
- b) Van Gieson's stain for collagen fibers (Singh and Sulochana, 1996).
- c) Masson's trichrome method for collagen fibers (Luna, 1968).
- d) Silver impregnation stain for Reticular fibers (Mukherjee, 1992).
- e) Wilder's method for reticulin (Luna, 1968).
- f) Verhoeff's stain for elastic fibers (Mukharjee, 1992).
- g) Crossman's modification of Mallory's triple stain for collagen and elastic fibers (Singh and Sulochana, 1996).
- h) Periodic acid Schiff's (PAS) stain for carbohydrate like glycogen, mucin and reticulin (Mukharjee, 1992).

The following micrometrical recordings were taken under simple microscope by micrometer scale after calibration at 10X ($1\mu = 15.38$ graduations) and 40 X power ($1\mu = 3.30$ graduation) magnifications.

1. Thickness of the stratum corneum (μm).
2. Thickness of the epidermis including stratum corneum (μm).
3. Number of Epidermal pegs per cm^2 of skin
4. Intensity of the melanin pigment

The numbers of epidermal peg, cm^2 was obtained by using formula of Benjamin *et al.* (1970).

The data collected was subjected to the statistical analysis as per the standard procedure suggested by Panse and Sukhatme (1967).

Results and Discussion

Epidermis was the outer most layer of skin composed of stratified squamous keratinized epithelium. It was found to be consisted of four layer viz. stratum basale, stratum spinosum, stratum granulosum and stratum corneum from inward to outward. However, stratum

lucidum and vasculature was found absent in epidermis in all breeds of cattle during both seasons under present study (Plate 1).

In agreement with the findings of Hole *et al.* (2008) in Red Kandhari cow and Aslan *et al.* (2004) in Zavot breed of cattle, the skin epidermis was stratified squamous type and composed of four sublayers viz. stratum basale, stratum spinosum, stratum granulosum and stratum corneum. Epidermal stratum lucidum was absent which was in contrast to the reports of Nagaraju *et al.* (2012), though the latter did not mention the region of presence of this sublayer.

Akers and Denbow (2008), Monteiro-Riviere (2007) and Samuelson (2007) in domestic animals reported that skin epidermis was of stratified squamous keratinized type. However, they mentioned the presence of stratum lucidum along with other epidermal sub layers. This difference with their reports may be due to their general histomorphological consideration on skin of domestic animals as they did not describe the region wise skin characteristics.

Stratum basale was the inner most epidermal layer, close to dermis. It was composed of single row of cuboidal to columnar cells with darkly stained oval or spherical nuclei, resting on the basement membrane. Sections stained by reticulin and PAS stain showed distinct reticular lamina of the basement membrane (Plate 17 – 20 and 114 - 117). The long axis of lining cells of this layer was oriented perpendicularly to the basement membrane. The cells of this layer showed mitotic figure in great extent in both seasons and breeds under present study.

These observations of the present study are in lines with findings reported by Hole *et al.* (2008) in Red Kandhari cow. Monteiro-Riviere (2007) and Samuelson (2007) reported similar organization and morphology of cells of stratum basale in domestic animals.

The melanocytes were observed as rounded cells with darkly stained spherical to elongated nuclei and pale cytoplasm scattered chiefly in between cells of stratum basale. The cytoplasm of melanocyte showed melanin granules, which were concentrated either on periphery or supranuclear in position. The mitotic activity was recorded among these cells (Plate 2). The melanocytes were also found in small extent scattered in between the stratum spinosum cells with considerably more amount of melanin granules as compared to the melanocyte of stratum basale in all breeds and seasons. Bloom and Fawcett (1971) stated that Langerhans cells in skin sections stained with gold chloride appeared blackened and revealed as stellate or dendritic cells. As gold chloride, one of component of Wilders method for reticulin stain, the Langerhans cells in sections stained by wilders method for reticulin during present study

exhibited similar morphology. These cells appeared as blackened cells and revealed as stellate or dendritic cells with processes extending in spaces among other surrounding cells of stratum basale and stratum spinosum (Plate 3) in all breeds and seasons.

In accordance with the observations recorded by Monteiro-Riviere (2007) and Samuelson (2007) in domestic animals, the presence of melanocytes and Langerhans cells in the basal region of the epidermis was revealed.

The stratum spinosum was the next layer above the stratum basale was usually found to be composed of two to three layers of oval to polyhedral cells with fairly stained nuclei. The cytoplasm showed melanin granules. Few melanocytes as well as Langerhan's cells were also observed scatter within cells of stratum spinosum layer in all breeds and seasons studied. This observation corroborates with the reports made by Hole *et al.* (2008) in Red Kandhari cow. Akers and Denbow (2008), Monteiro-Riviere (2007) and Samuelson (2007) in domestic animals reported similar observations regarding the morphology of cells of stratum spinosum. However, number of cell layers in this stratum reported by them varied from the numbers of cell layers of stratum spinosum in present study. This variation may be attributed to species variation.

Stratum granulosum was single cell layer in thickness in Deoni, Red Kandhari and Dangi, whereas it was two cell layers in thickness in Gaolao breed of cattle in both the seasons. The cells of this stratum were elongated to flattened in morphology with basophilic granular cytoplasm. However, many times their nuclei appeared indistinct because of abundant melanin granules in cytoplasm. The long axis of these cells was found aligned parallel to surface of skin. Similar observation was reported by Hole *et al.* (2008). They reported that stratum granulosum was single layer in thickness in Red Kandhari cow. However, two rows of stratum granulosum cells in Gaolao breed of cattle may be attributed to breed difference. The cells of stratum granulosum consist of protein filled keratohyaline granules that promotes cross linking of keratin, hydration and helps to prevent fluid loss from the body. This might be the reason for presence of two layers of stratum granulosum cells in epidermis of Gaolao cattle as this breed belongs to more hot climatic condition of Vidarbha region of Maharashtra as compared to other breeds of cattle under present study. Thus, two layers of stratum granulosum cells in epidermis of Gaolao cattle enables to prevent fluid loss from the body in hot climatic condition.

Stratum corneum was the outer most epidermal layer consisted of two to four layers of keratinized flattened cells in all breeds of cattle during summer season. However, it was

found to be composed of four to five cell layers in thickness during winter season in all breeds. In winter season, keratinized cells of stratum corneum were found loose and quite separated from each other (Plate -4). The cells of this layer were arranged parallel or slightly oblique in direction to the skin surface with pyknotic or absence of nuclei in all breeds and seasons, in present study.

In agreement with the observations recorded of Hole *et al.* (2008), similar range of cell layers were recorded in stratum corneum in Red Kandhari cattle. Similar morphological observations were also made by Monteiro-Riviere (2007) and Samuelson (2007) in domestic animals. They reported that the stratum corneum was the upper most epidermal layer composed of keratinized dead cells.

Increase in number of cell layers of stratum corneum during winter season might be correlated either to protect lower layers of skin from cold climatic condition or to xerosis of skin during winter season that prevents sloughing of superficial dead cells.

During present work, the melanin pigment was found distributed in the epidermis in all breeds of cattle. It was intensely distributed in the stratum basale and stratum spinosum layer of epidermis. However, intensity of melanin pigment was less in stratum granulosum and stratum corneum layer.

It was noticed that, melanin pigment distribution was more in Deoni and Red Kandhari breeds of cattle. It was found less in epidermis of Gaolao cattle as compared to other breeds of cattle. During present work, no seasonal variation in distribution of melanin pigment within different layer of epidermis was noticed in all breeds of cattle.

Epidermal pegs were the down growths of epidermis in to the underlying dermis. However, the number of cell layers of each epidermal stratum except stratum basale was found more at the region of epidermal pegs in all breeds and seasons in present study.

The mean values of average thickness of stratum corneum are presented in table 1. In the present study, the average thickness of stratum corneum during summer season was $11.94 \pm 0.34 \mu\text{m}$, $9.19 \pm 0.61 \mu\text{m}$, $11.7 \pm 0.39 \mu\text{m}$ and $13.23 \pm 0.49 \mu\text{m}$, whereas, during winter season it was found $14.52 \pm 0.36 \mu\text{m}$, $12.84 \pm 0.29 \mu\text{m}$, $14.88 \pm 0.29 \mu\text{m}$ and $16.21 \pm 0.11 \mu\text{m}$ in Deoni, Red Kandhari, Dangi and Gaolao breed of cattle, respectively. A significant increase in thickness of stratum corneum was recorded during winter season in all breed of cattle. The present observations are in agreement with Saxena *et al.* (1994) who reported increase in thickness of stratum corneum during winter season in JFL and FJL cross bred cattle.

Among breeds, average values of thickness of stratum corneum showed non-significant difference during summer season, whereas significantly different during winter season. However, non-significant difference was observed between Deoni and Dangi cattle during winter season.

In the present investigation, the average thickness of stratum corneum in all breeds of cattle except Red Kandhari cattle is observed to be higher than the thickness of stratum corneum of skin from back region in adult Gir cow as reported by Bhayani and Vyas (1991). Similarly, the average thickness of stratum corneum of Deoni cattle in both seasons and other breeds of cattle in summer season is within the range of reports made by Mugale and Bhosle (2002) in female Deoni cattle. However, in contrary to present observation they claimed decrease in thickness of stratum corneum during winter season. This variation in thickness of stratum corneum may be attributed to difference in breed and climatic conditions.

The increase in thickness of stratum corneum in winter season may be due to xerosis of skin during this season that may prevent the sloughing of superficial cells.

The mean values of average epidermal thickness are presented in table 2. The average thickness of epidermis during summer season was $27.20 \pm 1.09 \mu\text{m}$, $25.41 \pm 0.89 \mu\text{m}$, $26.58 \pm 2.09 \mu\text{m}$ and $32.20 \pm 0.73 \mu\text{m}$. Whereas, during winter season, it was $36.81 \pm 0.82 \mu\text{m}$, $36.11 \pm 0.82 \mu\text{m}$, $38.14 \pm 0.62 \mu\text{m}$ and $40.50 \pm 0.56 \mu\text{m}$ in Deoni, Red Kandhari, Dangi and Gaolao breeds of cattle, respectively. These recordings showed significant increase in the thickness of epidermis during winter season in all breeds of cattle. In the present study, the thickness of epidermis in Gaolao cattle differs significantly from other breeds of cattle during summer season. The values of average epidermal thickness during winter season showed significant difference among the breeds. However, non-significant variation was observed between Deoni and Red Kandhari cattle and between Dangi and Gaolao cattle in winter season.

During the present work, the average thickness of epidermis in all breeds of cattle except Gaolao cattle during summer season is within the range of reports made by Bhayani and Vyas (1991) in dorsal body region of Gir cattle. The variation in epidermal thickness between Gir and Gaolao cattle may be due to breed or seasonal difference.

Similarly, the thickness of epidermis during summer season in all breeds of cattle in present study is within the range reported by Saxena *et al.* (1994) in JFL and FJL cross breed cattle during summer season, whereas the thickness of epidermis recorded by them during winter season in both crossbreed cattle was found lower than the thickness of epidermis recorded during winter season in all breeds of cattle under present study. However, in support to the

present observations they stated that epidermis was significantly thinner during summer than winter season.

The average thickness of epidermis recorded in all breeds of cattle and season during present work is found to be lower than the epidermal thickness reported by Mugale and Bhosle (2002b) in skin of Deoni cattle at mid side body region, Hole *et al.* (2008) in Red Kandhari cow, Aslan *et al.* (2004) in Zavot breed of cattle, Muralidharan and Ramesh (2005) in Sindhi, Jersey and Jersey X.

Sindhi cross cow and Wang *et al.* (2012) in Thai native cow irrespective of age, body region and season. This variation in thickness of epidermis may be attributed to age, body region, seasonal variation and breed differences.

Table 1: Mean and SE of thickness of stratum corneum in the skin during summer and winter season in Deoni, Red Kandhari, Dangi and Gaolao cattle

Thickness of stratum corneum (μm)

Season	Deoni	Red Kandhari	Dangi	Gaolao
Summer	11.942 \pm 0.34	9.192 \pm 0.61	11.72 \pm 0.39	13.232 \pm 0.49
Winter	14.52 B1 \pm 0.36	12.84 C1 \pm 0.29	14.88 B1 \pm 0.29	16.21 A1 \pm 0.11

Table 2: Mean and SE of epidermal thickness of the skin summer and winter various seasons in Deoni, Red Kandhari, Dangi and Gaolao cattle

Thickness of epidermis (μm)

Season	Deoni	Red Kandhari	Dangi	Gaolao
Summer	27.20 B2 \pm 1.09	25.41 B2 \pm 0.89	26.58 B2 \pm 2.09	32.20 A2 \pm 0.73
Winter	36.81 B1 \pm 0.82	36.11 B1 \pm 0.82	38.14 A1 \pm 0.62	40.50 A1 \pm 0.56



PLATE 1: Photomicrograph showing epidermis of Deoni cattle during summer season

- A. Basement membrane
 - B. Stratum basale
 - C. Stratum spinosum
 - D. Stratum granulosum
 - E. Stratum corneum
 - F. Melanocytes
 - G. Langerhans cell
 - H. Melanin pigment
- (Haematoxylin and Eosin, X 1000)

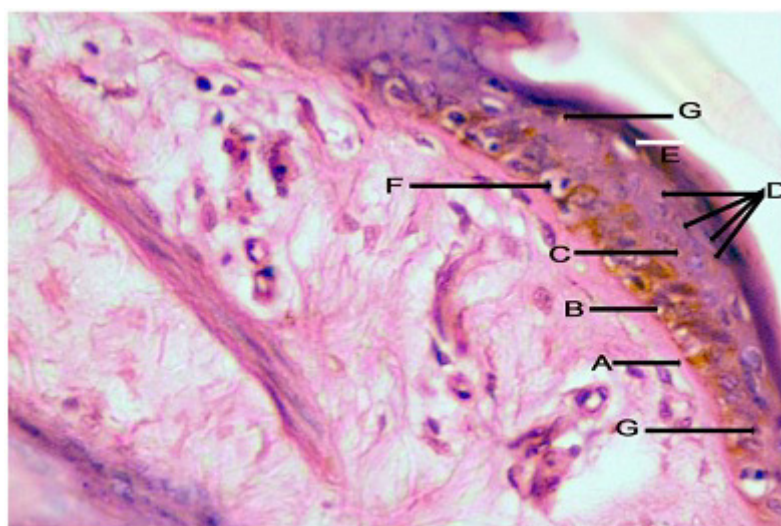


PLATE 2: Photomicrograph showing epidermis of Gaolao cattle during winter season

- | | | |
|----------------------|-----------------------|--------------------|
| A. Basement membrane | B. Stratum basale | G. Langerhans cell |
| C. Stratum spinosum | D. Stratum granulosum | H. Melanin pigment |
| E. Stratum corneum | F. Melanocytes | |
- (Haematoxylin and Eosin, X 1000)

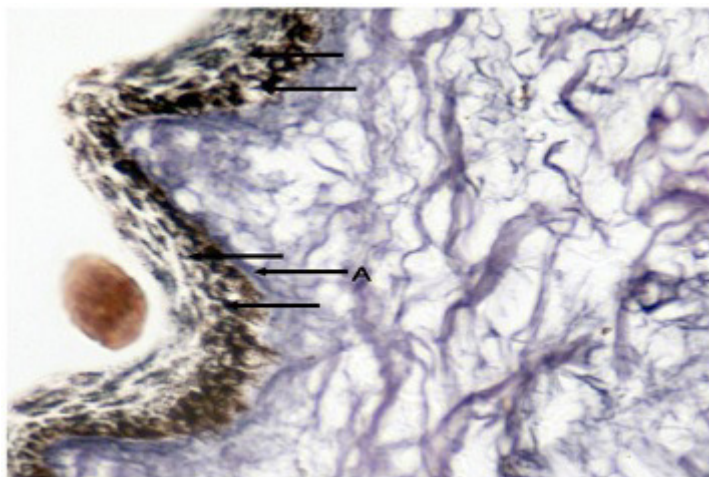


PLATE 3: Photomicrograph of skin of Deoni cattle during summer season

Arrow showing Langerhans cells in epidermis.

A. Reticular lamina of basement membrane

(Wilder's method for reticulin, X 1000)

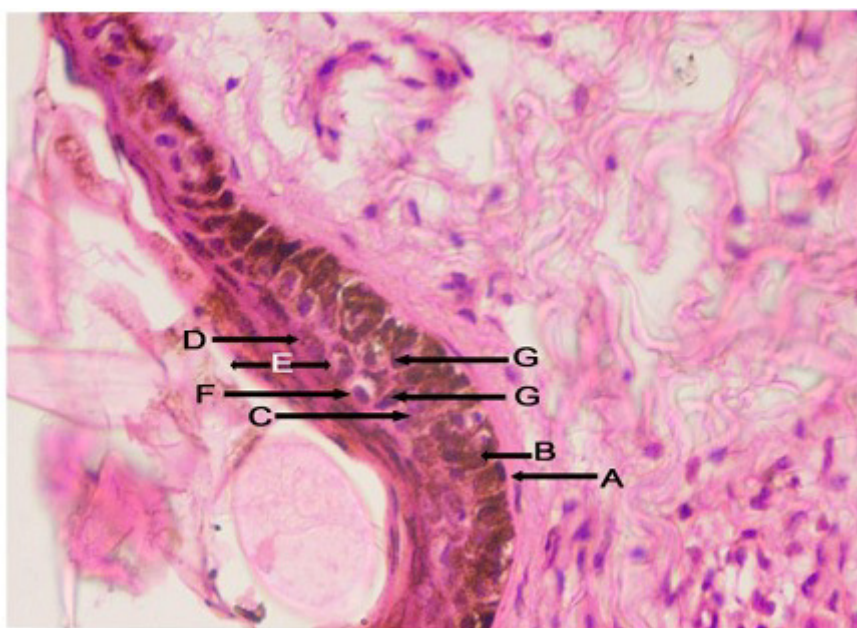


PLATE 4: Photomicrograph showing epidermis of Deoni cattle during winter season

A. Basement membrane

B. Stratum basale

C. Stratum spinosum

D. Stratum granulosum

E. Stratum corneum

F. Melanocytes

G. Langerhans cell

H. Melanin pigment

(Haematoxylin and Eosin, X 1000)

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